Note on the Cytology of Calothrix fusca.

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With Plate 32.

While studying the cytology of several Trichobacterinæ I came across a representative of the group of Cyanophyceæ, the study of which may cast perhaps some light on the question of relationship between Cyanophyceæ and Bacteria.

I found Calothrix fusca in aquaria among large quantities of Glœocapsa; I never found it not associated with those algæ. It seems not impossible that a symbiotic relationship exists between those two algæ, a relationship which would be obviously beneficial to Calothrix, this species being more or less deprived of chlorophyll. This question, however, must remain for the while a mere hypothesis, because I was not able to study the question more thoroughly.

The dimensions of the cells are very variable. At the end of the cell-filaments the cells are rather short (from $3{\cdot}6\,\mu$—$7{\cdot}2\,\mu$), but lengthen towards the base (from $7{\cdot}8\,\mu$—$10{\cdot}8\,\mu$); their breadth is from $3{\cdot}6\,\mu$—$7{\cdot}2\,\mu$. The filaments which are enclosed in thick hyaline sheaths are pseudo-ramified. Each pseudo-ramification possesses at its base a heterocyst and some concave cells.

To study the cytological details the cell-filaments were fixed in Pfeiffer's solution, washed in alcohol 60 per cent., after the ordinary passages through alcohol embedded in paraffin (52°), and cut in sections from 4—6 μ thick. The sections were washed in xylol and stained with iron-hematoxylin (Heidenhain).

I shall not enumerate here the different papers which have been published about the cytology of Cyanophyceæ. For reference to them the very complete works of Kohl,[1] Fischer,[2] and Guilliermond [3] may be consulted. According to Guilliermond, who recently studied various representatives of this group, the central mass of stainable matter (the "central body" of Bütschli[4]) is composed of chromatin which is supported by an achromatic substratum of alveolar structure; the whole central body is to be regarded as a primordial nucleus, a view which was already held by Bütschli and his followers. A contrary opinion is upheld by A. Fischer, who does not believe in the nuclear nature of the central body; according to this author it represents only the central part of the cytoplasma free from chlorophyll. The chromatophil bodies within it consist of a peculiar hydrocarbon called "anabænine." His strongest argument against the chromatic nature of those granules consists in the fact that they are dissolved in water. It must be remembered, however, that this argument no longer holds good, since Oes [5] showed that the chromosomes of Spirogyra are equally dissolved in water. Kohl (loc. cit.) showed that the chromatophil granules of the central body give all the characteristic reactions of true chromatin. I also have carefully examined the microchemical reactions of those granules, and have arrived at the same conclusion as Kohl's. I do not describe them here, since Kohl has done this *in extenso*. There cannot consequently be the least doubt that the granules within the central body

[1] Kohl, 'Über der Organisation und Physiologie der Cyanophyceen,' Jena, 1903.

[2] Fischer, 'Botan. Zeitung,' 1905.

[3] Guilliermond, 'Revue général de botanique,' 1907.

[4] Bütschli, 'Über die Bauder Cyanophyceen und Bacterien,' Leipzig, 1826.

[5] Oes, 'Botan. Zeitung,' 1908.

consist of real chromatin. I also observed in the cells of Calothrix fusca the metachromatic granules (volutine granules); they always were found in the central body.

The cells of Calothrix fusca do not possess a well developed chromatophore, surrounding the central body, as is found in other members of the Cyanophyceæ. Often the cells are not at all green coloured, and when this is the case the green colour is diffusely spread throughout the cell without a well-marked differentiation between coloured and non-coloured cytoplasma. This is perhaps the reason why the central body of Calothrix fusca is never so compactly built as in other Cyanophyceæ, and why it becomes so easily diffuse.

The cytoplasma of the young cells (at the end of the filaments) contains few or no inclusions, and has an alveolar structure (Pl. 32, figs. 1—5) which is not always distinctly visible. The central body is generally of normal shape. It is formed of an achromatic substratum (which, however, stains more deeply than the surrounding cytoplasma), in which are embedded the chromatic granules and filaments. The achromatic substratum determines the form of the central body; this form is rather variable, often the central body is star-shaped, and resembles the same organ of Tolypothrix lanata described by Kohl (loc. cit.). The chromatin is not always distributed throughout the whole central body; often several parts of it are free from chromatin (Pl. 32, fig. 5). Such specimens are very favourable for the study of the relation between the achromatic substratum of the central body and the surrounding cytoplasma. Both have an alveolar structure, and by carefully examining the places where cytoplasma and achromatic substratum come together, one can often observe that the septa of the cytoplasmic alveoli are continued without interruption into those of the achromatic substratum, the only difference consisting in the different avidity with which stains are absorbed (Pl. 32, figs. 5, 6).

Cell division is performed in the ordinary way; in the middle of the cell an imperfect ring-shaped transverse membrane is formed, which becomes afterwards closed. The central body divides by simple fissure, chromosome-like masses of chromatin not being found as is the case in other Cyanophyceæ (Kohl, Guilliermond).

Often it can be observed that the central body loses more and more its ordinary shape. It becomes elongated with more or less developed ramifications; often a slight curvature or zigzag form is to be observed (Pl. 32, figs. 2, 6, 7, 8 *c*), After carefully staining, one can always observe that the central body is normally formed by its two components, the chromatin and the chromatic substratum. In other specimens, however, the distinction between cytoplasma and achromatic substratum becomes more and more indistinct, and it is impossible at last to trace a distinction between the two (Pl. 32, figs. 8 *d*, *e*, 9). The chromatin is in such cases spread diffusely throughout the whole cell. The protoplasma of the latter is built after the ordinary pattern. The cells resemble very much those of some sporogenic bacteria, recently described by Guilliermond.[1]

The dissolution of the central body described here was also observed by Guilliermond (loc. cit.) in Scytonema cincinnatum, but it occurred there only in old vacuolated cells, so it is highly probable that the dissolution was of a pathologic origin. This, however, cannot be the case in Calothrix fusca, as very young cells (at the ends of young cell-filaments) show already this phenomenon (Pl. 32, fig. 8). In grown-up cells there appear in the cytoplasma large hyaline granules. They surround the central body at first, and seem afterwards to invade the latter, so causing its dissolution. I was at first deluded by this phenomenon, thinking that the dissolution of the central body had a purely mechanical cause, due to an auto-destruction by the formation of the hyaline granules. But a closer observation made clear that the diffusion of chromatin is equally found in cells which are not provided with hyaline granules (Pl. 32, figs. 8, 9), so

[1] Guilliermond, 'Arch. f. Prot. kunde,' 1908.

the destruction of the central body has not a merely mechanical cause.

I vainly tried to make out the chemical nature of these hyaline granules. They are not identical with the "cyanophycinkörnchen" of the German authors, nor do they consist of fat. They are only a little to be stained with eosine and carbolic fuchsin, they are dissolved in diluted acids and in pepsine, not in diluted alkalies.

The changes in cell-structure in the microtome sections were controlled by the study of toto-stained preparations. After fixation the cells were placed on a cover-glass, and were stained and imbedded in the ordinary way. The normal central bodies had the same aspect as in the sections (Pl. 32, fig. 10). The beginning of dissolution of the central body was also very clearly to be seen (Pl. 32, fig. 12) in these preparations, also the cells with diffuse chromatin (Pl. 32, fig. 11). Generally the distinction between the protoplasma of the central body and the surrounding parts was not very clearly to be seen. Except this point the toto-preparations had the same value as the sections.

I will now shortly discuss the results of the observations described here. The normally built central body of Calothrix fusca contains chromatin granules imbedded in the alveoli of the plasma of the central body (the "achromatic substratum"). The latter is easily differentiated from the cytoplasma; the alveoli of the latter are the continuation of those of the achromatic substratum. I think therefore with Guilliermond that the central body of the Cyanophyceæ must be regarded as a primordial nucleus, the difference of cytoplasma and nuclear plasma already existing, but being not yet very distinctly marked. Under certain circumstances, unknown to me, the central body becomes at first irregularly shaped (in this stage the central body resembles strikingly the "diffuse nuclei" of Opalinopsis and Foettingeria), after which the difference between cytoplasma and nuclear plasma (s. achromatic substratum) disappears, and the chromatin granules are spread throughout the whole cell.

The stages with diffuse chromatin resemble very much the Bacteria with chromatic granules spread throughout the protoplasma. There is not yet differentiation between cytoplasma and nuclear plasma. Other forms of Bacteria, with their chromatin condensed into a more or less compact central mass (Sphærotilus [Swellengrebel[1]], Bac. spirogyra [Dobell[2]]), find their match in those forms of Calothrix fusca, where a well-marked differentiation between cytoplasma and nuclear plasma does not yet exist, but where the chromatin is no longer spread throughout the whole cell, but takes a central position (Pl. 32, fig. 8 *c*). The stage with a well developed central body is not yet found in the group of Bacteria.

Bütschli (loc. cit.) has already observed the resemblance of the structure of Cyanophyceæ and larger Bacteria, the latter showing a central agglomeration of chromatin suggesting a central body. I think that the stages with diffuse chromatin come much nearer to the structures described in Bacteria, and that these observations may aid to support the view concerning the relationship between Cyanophyceæ and Bacteria.

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EXPLANATION OF PLATE 32,

Illustrating Dr. N. H. Swellengrebel's paper "Note on the Cytology of Calothrix fusca."

(Drawings made under a Zeiss 2 mm. homog. oil immersion apochromatic comp. oc. 18.)

Figs. 1—9.—Sections from 4—6 μ thick.

Figs. 1 and 3.—Three cells showing a well-developed central body with differentiation into chromatin and achromatic substratum.

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[1] Swellengrebel, 'C. R. Soc. de biol.,' Juin, 1908.

[2] C. C. Dobell, 'Quart. Journ. Micros. Science,' vol. 53, May, 1909.

Figs. 2 and 4.—Central body in the act of becoming diffuse.

Fig. 5.—Showing the star-shaped achromatic part of the central body.

Fig. 6.—Idem. Achromatic part zigzag shaped.

Fig. 7.—As fig. 5, but achromatic substratum no longer visible.

Fig. 8.—Showing the different stages of dissociation of the central body.

a, b. Normal central body.

c. Central body become diffuse. Achromatic substratum no longer differentiated.

d, e. Complete dissolution of the central body. Chromatin in the form of granules and filaments spread throughout the protoplasma.

Fig. 9.—Same stages as fig. 8*d* and 8*e*.

Figs. 10—12.—Preparations of cell-filaments stained in toto.

The figures show the same peculiarities as the microtome sections.